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Hypo- or Hyper-Hippo: A Balancing Act with bHLH Transcription Factors

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Balancing cell growth with differentiation is essential for tissue integrity. In this issue of *Developmental Cell*, Wang and Baker (2015) demonstrate unsuspected cross-talk between bHLH transcription factors, important regulators of organogenesis, with the Hippo tumor suppressor pathway to ensure that inappropriately differentiating cells are eliminated during development.

Tissue homeostasis requires a delicate balance between cell proliferation and survival with differentiation and death, with disruptions in this balance underlying numerous developmental defects and cancer. Hence, the cell has evolved multiple checks and balances to avoid disastrous consequences. How these checks and balances are integrated, interpreted, and regulated by the cell, however, is still largely unknown. In the current issue of *Developmental Cell*, Wang and Baker (2015) address this important question, revealing that the Id helix-loop-helix (HLH) transcription factors, which are known to promote proliferation and function as oncogenes (Lasorella et al., 2014), cross-talk with the Hippo tumor suppressor pathway as part of a mechanism that ensures that cells undergoing inappropriate differentiation are properly eliminated. This newly uncovered surveillance system provides important insights into control mechanisms that safeguard tissue homeostasis. It also represents one of the first examples of a continual physiological role for Id proteins and Hippo signaling in maintaining organ integrity.

The Hippo signaling cascade is a highly conserved tumor suppressor pathway important for inhibiting cell proliferation and survival (Figure 1A) (Harvey et al., 2013). Hippo (Mst1,2 in vertebrates) is a serine/threonine kinase that, in conjunction with the adaptor proteins Salvador/WW45 and Mats/NOBKL1A/B, phosphorylates another kinase, Warts (Wts)/Lats1,2. Hence, the Hippo pathway is also referred to as the Salvador–Warts–Hippo (SWH) pathway. Wts/Lats is the main effector kinase in the pathway, executing this role by preventing the oncogenic transcriptional co-activator Yki/Yap/TAZ from promoting the expression of cell cycle and anti-apoptotic genes. Besides these core components of the pathway, a number of "accessory" factors regulate Hippo signaling activity, many of which suggest that SWH activity is regulated through mechanical force via cell junctions and the actin cytoskeleton (Gaspar and Tapon, 2014). One such factor is the FERM domain protein, Expanded (Ex), which is recruited to the cell membrane by the apical membrane protein Crumbs. Ex forms a stabilizing feedback system with Yki to ensure limited Hippo signaling and prevent tissue overgrowth. First, through a process not yet clearly defined, Ex activates the SWH kinase cascade, thereby indirectly preventing Yki's nuclear accumulation. Second, Ex can physically associate with non-phosphorylated Yki, resulting in Yki's sequestration at the plasma membrane. And third, the *ex* gene is a direct transcriptional target of Yki, so that minimal Yki activity promotes its own inactivation by activating *ex* and the SWH pathway. Together, this Ex-Yki feedback helps to ensure homeostasis between cell proliferation and cell death.

While the importance of Hippo signaling in preventing tumorigenesis is undisputed, most studies of this pathway have been performed using loss- and gain-of-function studies, which forcefully tip the balance of the Hippo pathway toward one side or the other. However, our understanding of how intermediate levels of Hippo signaling are achieved and function under physiological conditions is still quite limited. The studies by Wang and Baker (2015) begin to lend insight into this open question. Notably, this work arose not from studies designed to understand Hippo signaling, but instead from studies designed to define the growth-regulatory functions of another family of proteins: the HLH superfamily of transcription factors.

Basic HLH (bHLH) factors are well-recognized for their roles in organogenesis and are critical for coordinating cell-cycle arrest with the onset of differentiation (Lasorella et al., 2014; Huang et al., 2014). The best-known members in this family of proteins are tissue-specific (e.g., MyoD, NeuroD), but these bHLHs require heterodimerization with more widely expressed E box binding factors, or E proteins (e.g., vertebrate E12 or E47/TCF3 and *Drosophila* Daughterless [Da]), to regulate gene expression. The Id proteins, in contrast, are non-DNA-binding HLH factors that heterodimerize with bHLHs to inhibit their gene regulatory activity. *Drosophila* encodes a single E protein (Da) and a single Id protein, called *extramacrochaetae* (*emc*). Da and Emc form a negative regulatory loop, as Da promotes its own expression as well as that of emc, while Emc represses both *da* gene expression and Da function (Bhattacharya and Baker, 2011). Accordingly, *emc* loss-of-function and *da* gain-of-function studies produce similar phenotypes: reduced organ size, cell death, and ectopic neurogenesis (Figure 1B). Thus, much like the Hippo pathway, Da and Emc maintain a homeostatic balance

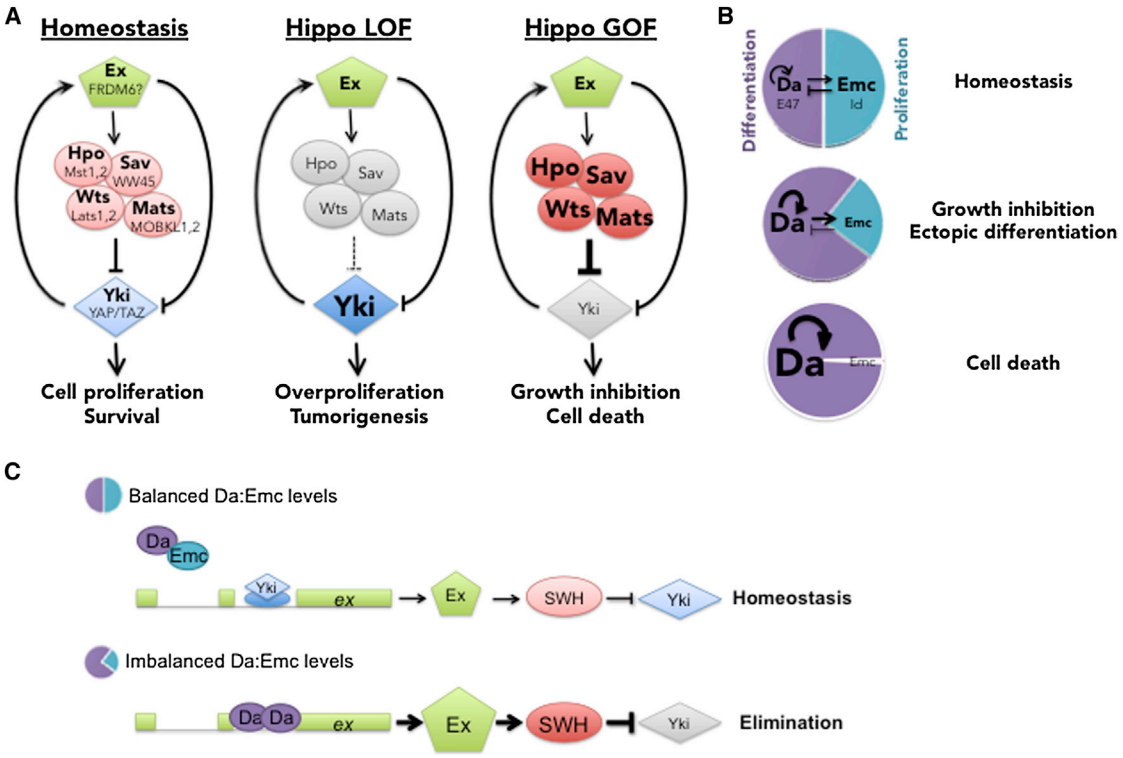

Figure 1. Roles for the Hippo Pathway and bHLH Factors in Regulating Cell Growth and Differentiation
(A) Diagram of the core Hippo pathway in wild-type and mutant conditions, including Yki-Expanded (Ex) feedback. (B) Phenotypic outcomes from altering Da:Emc ratios. (C) Model for bHLH-mediated activation of the Hippo pathway. Vertebrate orthologs are listed below *Drosophila* names in first diagram in (A) and (B). See text for more details.

to promote normal cell proliferation and differentiation.

In an effort to understand Da/Emc-dependent cell growth regulation, Wang and Baker (2015) performed a modifier screen in *Drosophila* overexpressing Da. As reported, this screen uncovered some, but not all, Hippo pathway components as necessary for Da-driven growth inhibition. Because Da is a transcription factor, they probed whether this regulation occurred through changes in Hippo pathway gene expression, revealing that Da activates the expression of *ex* (Figure 1C). Through enhancer mapping and chromatin immunoprecipitation (ChIP) studies, they refined the Da-response element to a 658 bp enhancer within the *ex* locus that requires Da binding sites (E boxes) for its activation. These data suggest that increased Da (and/or decreased Emc) inhibits growth through Ex-dependent activation of the SWH pathway and the ensuing negative control of Yki. Consistent with *ex* being downstream of Da, eliminating *ex* from imaginal disc cells prevents Da-dependent growth inhibition. Surprisingly, however, Da-dependent neurogenesis is strongly increased under these same conditions. Thus, the authors propose a model in which cells that acquire an inappropriate balance of Da:Emc levels during development activate the Hippo pathway, leading to their elimination, thereby preventing excess neurogenesis. Importantly, this growth suppression network appears to function independently of Crumbs-dependent membrane recruitment. Combined, these data suggest that an imbalance of Id:bHLH ratios leads to cell contact-independent activation of the Hippo pathway to eliminate cells with an inappropriate developmental fate. This work also emphasizes the importance of coordinated changes in the stoichiometry to both bHLH factors and the Hippo pathway for their ability to maintain tissue integrity.

It will be important and exciting to probe in further depth whether this mechanism is conserved. This possibility, however, hinges on the still-open question of whether Ex is functionally conserved. FRDM6, or Willin, shares the highest sequence similarity with *Drosophila* Ex but lacks the domain in Ex that mediates interactions with Yki and promotes SWH activity, questioning its functional homology (Bossuyt et al., 2014). Evidence for or against this is inconsistent. For example, although human Ex displays tumor suppressor activity in cultured cells, its ability to activate the Hippo pathway is controversial and may be context dependent (Visser-Grieve et al., 2012; Angus et al., 2012). It is therefore tempting to speculate that bHLH-mediated activation provides such context. Thus, this recent work by Wang and Baker will provide a valuable paradigm for future comparative studies on Id and SWH pathway-dependent cell growth and differentiation events.

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REFERENCES

Angus, L., Moleirinho, S., Herron, L., Sinha, A., Zhang, X., Niestrata, M., Dholakia, K., Prystowsky, M.B., Harvey, K.F., Reynolds, P.A., and Gunn-Moore, F.J. (2012). Oncogene *31*, 238–250.

Bhattacharya, A., and Baker, N.E. (2011). Cell *147*, 881–892.

Bossuyt, W., Chen, C.-L., Chen, Q., Sudol, M., McNeill, H., Pan, D., Kopp, A., and Halder, G. (2014). Oncogene *33*, 1218–1228.

Gaspar, P., and Tapon, N. (2014). Curr. Opin. Cell Biol. *31C*, 74–83.

Harvey, K.F., Zhang, X., and Thomas, D.M. (2013). Nat. Rev. Cancer *13*, 246–257.

Huang, C., Chan, J.A., and Schuurmans, C. (2014). Curr. Top. Dev. Biol. *110*, 75–127.

Lasorella, A., Benezra, R., and Iavarone, A. (2014). Nat. Rev. Cancer *14*, 77–91.

Visser-Grieve, S., Hao, Y., and Yang, X. (2012). Oncogene *31*, 1189–1195.

Wang, L.-H., and Baker, N.E. (2015). Dev. Cell *32*, this issue, 191–202.

Enhancer Trafficking: Free Throws and Three-Pointers

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Accurate targeting of transcriptional enhancers to the correct promoter poses a significant information problem in higher eukaryotes. Enhancer-core promoter specificity may provide one solution. Reporting recently in *Nature*, Zabidi et al. (2014) uncover, via a genome-wide analysis approach, two general classes of enhancer-promoter interactions differentially regulating "housekeeping" versus "developmental" genes.

Transcriptional regulation in eukaryotes is directed by distally acting enhancers and proximal core promoters that contain motifs such as the TATA box and DPE, which interact with the general transcription machinery. In metazoan genomes, tens of thousands of enhancers must selectively interact with transcriptional start sites to direct accurate gene expression. Although enhancer-promoter proximity is an important simplifying constraint, with further control exerted by insulators and promoter targeting sequences, additional means ensure that *trans*-acting factors on enhancers interact with the correct basal promoters. Specificity in enhancer-core promoter interactions has been described in a few cases, most notably in *Drosophila*, but the generality of such interactions is unknown. Indeed, there is substantial evidence that many enhancers have the potential to activate a wide range of basal promoters (Kermekchiev et al., 1991).

The Stark group recently described a powerful new technique to identify transcriptional enhancers on a genome-wide scale (Arnold et al., 2013). Rather than relying on the identification of characteristic chromatin marks that are assumed, but not proven, to correspond to actual functional elements, their STARR-Seq method tests small genomic fragments inserted into transiently transfected reporters. The genomic elements are positioned in a downstream "enhancer-like" location that ensures the regulatory region will be transcribed together with the reporter, permitting identification of the *cis* element in high-throughput sequencing assays. Although the method is limited in that it does not reveal active "silencers" or complex, multi-component regulatory regions, it represents the cutting edge in making functional maps of *cis* elements.

The assay matches many enhancers to one core promoter sequence, and in the new study from the group, Zabidi et al. (2014) show that just which promoter is selected greatly influences the interpretation of the regulatory potential of the *Drosophila* genome. The authors use a core promoter sequence from a ribosomal protein gene (*RpS12*), representing a broadly expressed housekeeping gene, and a modified element derived from the tissue-specific *even-skipped* gene (*eve*) as a developmental core promoter. Significantly, the authors find that enhancers that activate the *RpS12* construct differ from those functioning with the modified *eve* promoter. Enhancers working with the *RpS12* core promoter typically lie close to transcriptional start site of the genes from which they were derived, while enhancers preferring the *eve* core promoter are typically more distal, reminiscent of the paradigmatic stripe enhancers of the *eve* locus.

Overall, the genes associated with the enhancers collected through this process fall into two broad categories. *Rps12*-sensitive enhancers are associated with what have been termed "housekeeping" genes, characterized by broad or ubiquitous expression (although their expression levels may vary in time or space). The *eve* core promoter collected enhancers of genes expressed in a more restricted fashion, hence termed "developmental" genes. It